Do unsaturated phosphoinositides mix with ordered phosphadidylcholine model membranes?

Antje Hermelink and Gerald Brezesinski[1]

Max Planck Institute of Colloids and Interfaces, Research Campus Golm, 14476 Potsdam, Germany

Abstract **Phosphoinositides have been shown to control membrane trafficking events by targeting proteins to specific cellular sites, which requires a tight regulation of phosphoinositide generation and turnover as well as a high degree of compartmentalization. To shed light on the processes that lead to the formation of phosphoinositide-enriched microdomains, mixed monolayers of phosphatidylcholine and dioleoyl-phosphatidylinositol (DOPtdIns) or dioleoyl-phosphatidylinositol-bisphosphate [DOPtdIns(4,5)P_2] were investigated by isothermal area/pressure measurements, Brewster angle microscopy, and grazing incidence X-ray diffraction. The results are consistent with a charge-dependent formation of phosphatidylinositol-containing tightly packed phases. DOPtdIns is capable of mixing partially with condensed 1,2-distearoyl-phosphatidylcholine (DSPC) and of forming mixed crystals that differ significantly from those formed by pure DSPC. DOPtdIns(4,5)P_2 in mixtures with DSPC is, to a much larger extent, phase separated. The observed phase separation of the highly charged DOPtdIns (4,5)P_2 is presumably water stabilized by electrostatic interactions and hydrogen bonding. In biological systems, an enzymatic phosphorylation of phosphatidylinositol in mixed domains may cause their insolubility in ordered phosphatidylcholine areas and lead to a cooperative reorganization of the host lipid membrane. This strong cooperative effect underlines the important role of PtdIns(4,5)P_2 in signal transduction processes and suggests that the ability of phosphoinositides to induce or reduce long-range interactions in phospholipid mixtures is crucial.**—Hermelink, A., and G. Brezesinski. **Do unsaturated phosphoinositides mix with ordered phosphadidylcholine model membranes?** ***J. Lipid Res.*** **2008.** 49: **1918–1925.**

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Views on how cell membranes are organized are presently changing. The lipid bilayer that constitutes these membranes is no longer understood as a homogeneous fluid. Instead, lipid assemblies, termed rafts, have been introduced to provide fluid-ordered platforms that segregate membrane components and dynamically compartmentalize membranes (1–4). These assemblies are thought to be composed mainly of sphingolipids and cholesterol in the outer leaflet, somehow connected to domains of unknown composition in the inner leaflet. Phosphoinositides are lipids appearing exclusively in the inner leaflet of the membrane, regulating numerous processes, including protein trafficking and signal transduction, and have shown to be highly sensitive to environmental ions (5).

Studies with lipid monolayers and bilayers have demonstrated that mixtures of lipids mimicking the composition of the outer leaflet of the plasma membrane exhibit liquid-liquid immiscibility and segregate into liquid-ordered and liquid-disordered domains. Sphingomyelin (SpM), which carries mostly saturated hydrocarbon chains, preferentially partitions with cholesterol into liquid-ordered phase domains, segregating from unsaturated phosphatidylcholines (PCs), which are the major constituents of domains of the liquid-disordered phase. The size of domains seems to vary greatly, depending on temperature, pressure, and composition. Wang and Silvius (6) studied mixtures of inner leaflet lipids together with cholesterol and could not detect formation of segregated liquid-ordered domains. Keller et al. (7), on the other hand, could see phase segregation when small amounts of SpM were present. Hydrogen bond formation between lipid headgroups has generally been identified as a source of lipid phase stabilization (8). For example, the pH-dependent analysis of dipalmitoylphosphatidic acid phase behavior revealed increased gel-phase stability (higher main-phase transition temperature) between the pKa1 and pKa2 of the phosphomonoester group. This strong mutual phosphatidic acid (PA) interaction was also evident in calorimetric studies of mixed PC/PA vesicles, which showed fluid/fluid immiscibility at pH 4 (9). The observed pH dependence of the mutual PA interaction was attributed to the fact that the presence of hydrogen-donating and hydrogen-accepting groups is required for the formation of a hydrogen bond network. Although this is fulfilled for a partially dissociated phosphomonoester

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[1] To whom correspondence should be addressed.
e-mail: brezesinski@mpikg.mpg.de

group (pKa1 < pH < pKa2), this requirement is not fulfilled for the fully protonated or deprotonated state. In the case of phosphoinositides, it is far more challenging to predict the mutual interaction, because three types of functional groups (phosphodiester, phosphomonoester, and hydroxyl groups) can potentially participate in hydrogen bond formation. Furthermore, the number and position of the phosphomonoester groups at the inositol ring is likely to impact the mutual phosphoinositide interaction.

This study is concerned with the physical-chemical characterization of mixed phospholipid monolayers formed by binary mixtures of 1,2-distearoyl-phosphatidylcholine (DSPC) with dioleoyl-phosphatidylinositol (DOPtdIns) or dioleoyl-phosphatidylinositol-bisphosphate [DOPtdIns(4,5)P_2]. The experiments are focused on the effect of the inositides on the structure of the condensed DSPC phase as the host lipid.

EXPERIMENTAL PROCEDURES

Materials

1,2-Dioleoyl-*sn*-glycero-3-phosphatidylinositol (DOPtdIns) and 1,2-dioleoyl-*sn*-glycero-3-[phosphatidylinositol-4,5-bisphosphate] [DOPtdIns(4,5)P_2] from Avanti Polar Lipids were used as received and dissolved in chloroform-methanol-water (65:35:6, v/v/v) (J. T. Baker; Deventer, Holland) to give a 1 mM stock solution. DSPC was obtained from Fluka and also used without further purification. The aqueous subphase consisted of 10 mM phosphate buffer (PB), pH 8, (Sigma-Aldrich; Steinheim, Germany). For all subphases, Millipore system-purified water with a resistivity of 18.2 MΩ·cm was used.

Langmuir film balance

The surface pressure/area isotherms (π/A) were recorded on a film balance from Riegler & Kirstein (R & K) GmbH (Potsdam, Germany). The surface pressure was determined by the Wilhelmy method using filter paper as Wilhelmy plate. After 20 min of solvent evaporation, the spreaded monolayers were compressed by means of a movable barrier with a velocity of 5 $Å^2 \cdot molecule^{-1} \cdot min^{-1}$ while the surface pressure and the area were continuously recorded. Each isotherm was reproduced at least three times. All measurements were conducted at 20°C.

Grazing incidence X-ray diffraction

Grazing incidence X-ray diffraction (GIXD) experiments were performed at the undulator beamline BW1 at HASYLAB, DESY (Hamburg, Germany) using the liquid-surface diffractometer. The incident angle, α_i, of the monochromatic beam was kept below the critical angle of total reflection for the air/water interface, $\alpha_c = 0.13°$, ($\alpha_i = 0.85 \cdot \alpha_c$), and the diffracted intensity was recorded as a function of the horizontal and vertical scattering angles $2\theta_{xy}$ and α_f, respectively, by a linear position-sensitive detector (OEM-100-M; Braun, Garching, Germany). A Soller collimator giving a horizontal resolution of 0.008 $Å^{-1}$ was placed in front of the detector, and the entire system (collimator and detector) was rotated to set the horizontal scattering angle. The X-ray beam was made monochromatic ($\lambda \approx 1.3$ Å) by a beryllium (002) crystal. The out-of-plane [$Q_z \approx (2\pi/\lambda) \cdot \sin(\alpha_f)$] and in-plane [$Q_{xy} \approx (4\pi/\lambda) \cdot \sin(2\theta_{xy}/2)$] components of the scattering vector Q provide information about the laterally periodic structures of the monolayer in terms of lattice parameters, tilt angle, tilt direction, and lattice distortion. Detailed descriptions of the experimental set-up and the theoretical background of the GIXD experiment can be found in the literature (10–12).

Brewster angle microscopy

The morphology of the monolayer was visualized with a Brewster angle microscope (BAM1) (Nanofilm Technology; Göttingen, Germany) mounted on a Langmuir film balance (R & K). A helium-neon laser (10 mW) produces light with a wavelength of 632.8 nm. After passing a polarizer, the p-polarized light is directed to the water surface under the Brewster angle. The diameter of the beam is 0.68 mm. The trough is mounted on a X-Y translation table (Märzhäuser; Wetzlar, Germany), which is placed on an anti-vibrational table (JAS; Affoltern, Switzerland) (13, 14). Image processing software was used to correct the Brewster angle microscopy (BAM) images for the distortion due to the observation at the Brewster angle. BAM is sensitive to changes in layer thickness and orientation of the aliphatic chains. The resolution of the BAM1 is about 4 μm. The images shown here are 500·500 μm^2 in size (scale bars indicated).

RESULTS

Partial phase separation in binary mixtures of DSPC and DOPtdIns

π/A isotherms of a pure DSPC monolayer, a pure DOPtdIns, and binary mixtures of both in different molar ratios (molar fraction of DOPtdIns x_{PI} = 0.05, 0.25, 0.45, and 0.5), measured at 20°C on 10 mM PB with pH 8, are shown in **Fig. 1** (left). The thermodynamic analysis of mixed phospholipid Langmuir monolayers provides information about miscibility tendencies of the components. The isotherm of pure DSPC (x_{PI} = 0) shows a condensed phase at all surface pressures investigated. This monolayer is stable up to high lateral pressures (collapse occurs around 60 $mN \cdot m^{-1}$, depending on the subphase composition and the compression speed). Due to the double bonds in both fatty acid chains, the DOPtdIns film exhibits only a disordered fluid phase with a corresponding low collapse pressure at approximately 30 $mN \cdot m^{-1}$. In the binary mixtures, the film becomes progressively more expanded with increasing molar percentage of DOPtdIns, and for equivalent pressures, the average molecular area shifts to larger values (Fig. 1 right). A linear increase of the molecular area on going from DSPC to DOPtdIns (dashed lines in Fig. 1 right) can be expected for ideally mixed or completely demixed binary systems. For lateral pressures up to 20 $mN \cdot m^{-1}$, the addition of DOPtdIns leads to positive deviations from the linear relation except for mole fractions around 0.35. Positive deviations of the mean molecular area from linearity indicate a positive sign for the excess free energy of mixing ΔG^{exc} denoting repulsive interactions between unlike molecules in the mixtures. For mixtures containing more than 35 mol% DOPtdIns, the isotherm changes its slope around a surface pressure of 20 $mN \cdot m^{-1}$, indicating either a structure change within the mixed DSPC/DOPtdIns monolayer, a partial collapse of the DOPtdIns-rich domains, or a surface pressure-induced change in miscibility of the two compounds. At high surface pressure values, the slope of the isotherms (and therefore

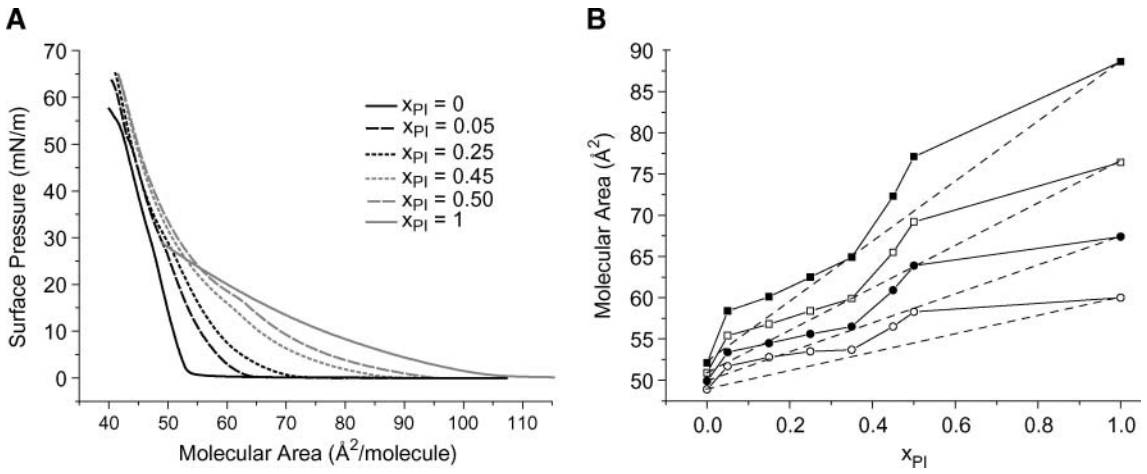

Fig. 1. A: π/A isotherms of 1,2-distearoyl-phosphatidylcholine (DSPC) on 10 mM phosphate buffer (PB), pH 8, containing dioleoyl-phosphatidylinositol (DOPtdIns) (the mole fractions x_{PI} of DOPtdIns are indicated). B: Average molecular area in dependence on the molar ratio of the two compounds at 5 $mN \cdot m^{-1}$ (closed cubes), 10 $mN \cdot m^{-1}$ (open cubes), 15 $mN \cdot m^{-1}$ (closed circles), and 20 $mN \cdot m^{-1}$ (open circles). The dashed lines represent the linear behavior for a completely demixed or ideally mixed binary system.

the compressibility of the monolayer) is almost the same as observed in the pure DSPC isotherm. This can be attributed to the fact that DOPtdIns is not able to form stable monolayers at high surface pressure values. The slight deviations from linearity seen in Fig. 1 (right) indicate already slight repulsive interactions between the two compounds. Large regions of immiscibility can be expected. This can be understood based on the miscibility selection rules of liquid crystals (15), because both compounds form different monolayer structures.

To confirm the demixing in the DSPC/DOPtdIns monolayer, the mixed films were observed by BAM. BAM images recorded on compression of DSPC/DOPtdIns ($x_{PI} = 0.25$) monolayers are presented in **Fig. 2**. At all pressures investigated, the monolayer appears inhomogeneous, indicating phase separation. Because fluid films have a smaller

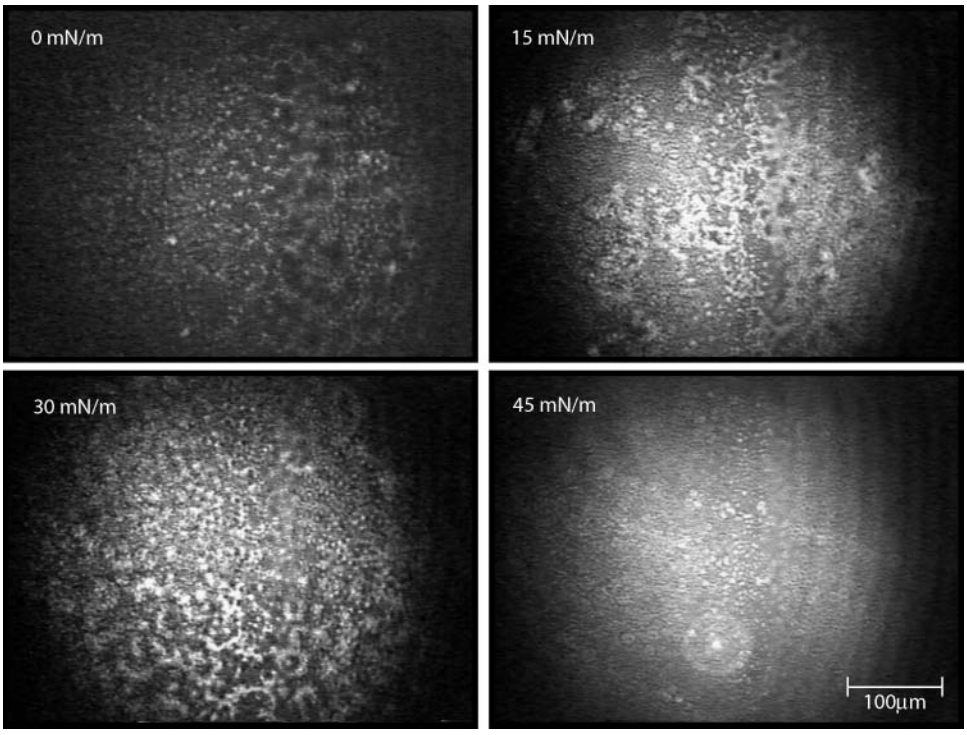

Fig. 2. Brewster angle microscopy (BAM) images of a 3:1 DSPC/DOPtdIns ($x_{PI} = 0.25$) mixed monolayer on 10 mM PB, pH 8, at 20°C. Surface pressures and scale bar are indicated. The corresponding isotherm can be found in Fig. 1.

thickness compared with condensed phases, the darker areas in the BAM image were attributed to fluid regions containing mainly DOPtdIns. Although the film does not change basically its morphology on compression, the brighter areas in the images become more dominant, indicating a decrease in the area occupied by the fluid phase. This can be easily understood in terms of the higher compressibility of a fluid phase.

GIXD was used to determine the extent to which the addition of DOPtdIns affects the structure of the condensed DSPC monolayer. As indicated by the surface pressure/area isotherms, DOPtdIns exhibits only a liquid-expanded phase and therefore shows no long-range lateral ordering. In contrast, DSPC exhibits only a condensed phase: The diffraction pattern of this phase is characterized by two diffraction peaks at all pressures up to the collapse. One Bragg peak is located at zero Q_z and the second one at values of $Q_z > 0$. Such an intensity distribution is characteristic for a monolayer phase named L_2 with a centered rectangular structure and chains tilted toward the nearest neighbors (NN) (16). The tilt angle is relatively large due to the mismatch of area requirements of the strongly hydrated headgroup and the two chains in all-*trans* conformation and decreases with increasing surface pressure. **Fig. 3** shows selected contour plots of the corrected X-ray intensities as a function of the in-plane, Q_{xy}, and out-of-plane, Q_z, components of the scattering vector Q for the pure DSPC (left column) and mixed DSPC/DOPtdIns (3:1, $x_{PI} = 0.25$) (middle column) monolayers. At low pressures, the mixed monolayer exhibits also a centered rectangular structure with NN tilt. However, the tilt angle in the mixture is clearly reduced compared with pure DSPC. At higher surface pressures, a diffraction pattern with only one Bragg peak can be seen that was not observed in pure DSPC monolayers. This pattern indicates that the aliphatic tails are oriented upright and arranged in a hexagonal lattice (LS phase). This pronounced effect of DOPtdIns on the monolayer structure depends on the molar ratio be-

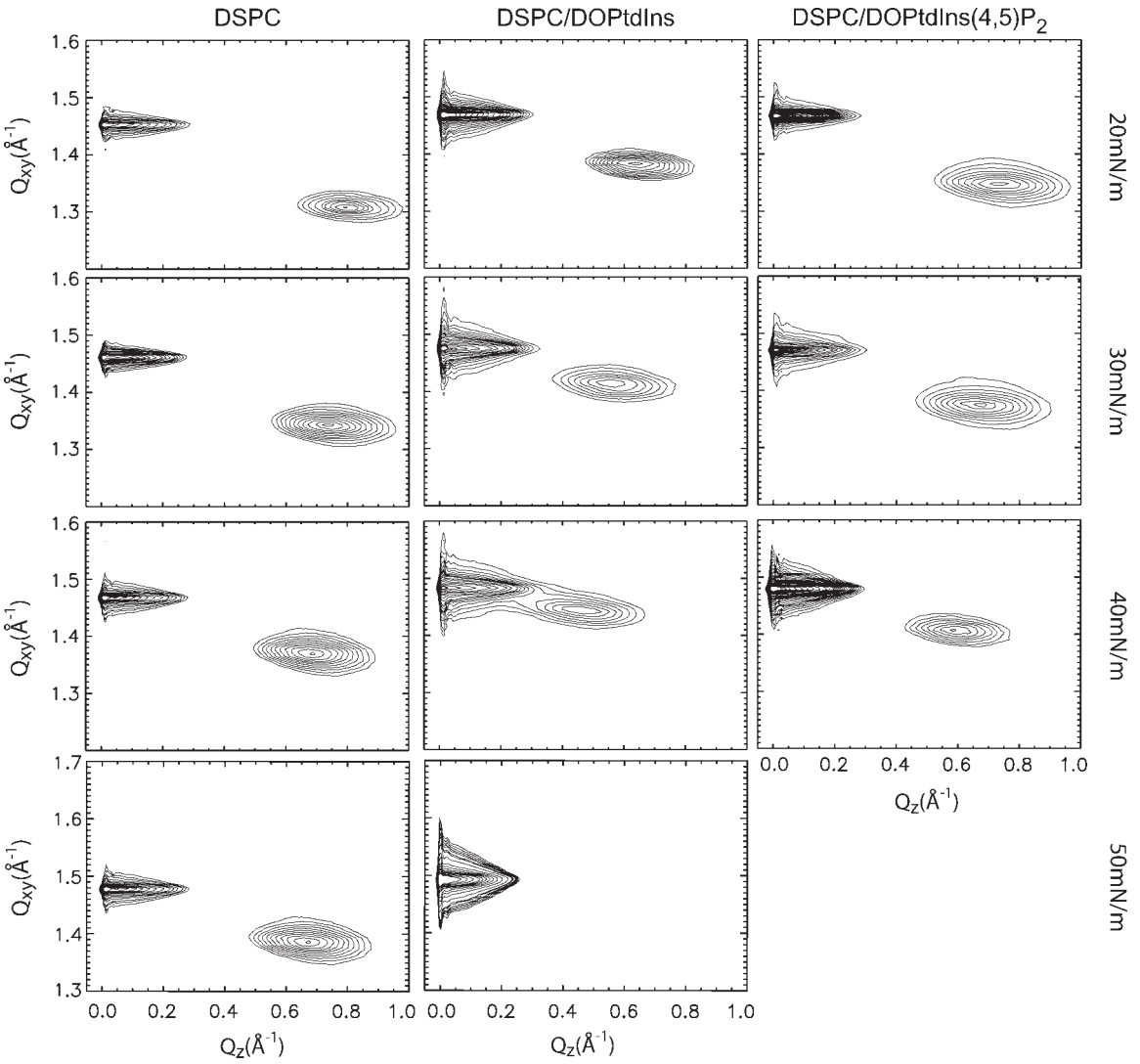

Fig. 3. Contour plots of the corrected X-ray intensities as a function of the in-plane component Q_{xy} and the out-of-plane component Q_z of the scattering vector Q of a DSPC (left), a DSPC/DOPtdIns ($x_{PI} = 0.35$, middle) and a DSPC/dioleoyl-phosphatidylinositol-bisphosphate [DOPtdIns(4,5)P_2] ($x_{PIP2} = 0.35$, right) mixture at 20°C and the indicated surface pressures.

tween the two components. For the 4:1 mixture of DSPC and DOPtdIns (x_{PI} = 0.20), the decrease of the tilt angle was observed as well, but the hexagonal nontilted phase could not be reached (**Fig. 4** left). There is no doubt that the two components forming different phase structures cannot be completely miscible; this has been already concluded from the isotherm and BAM measurements. However, the amount of DOPtdIns, which is obviously mixed with DSPC, changes the monolayer structure of the host lipid DSPC drastically. The amount of lattice-integrated DOPtdIns must be, however, quite small, thus indicating that one DOPtdIns molecule can influence the packing of numerous neighboring DSPC molecules and induce a higher packing density. In order to achieve this more-efficient packing, the DOPtdIns molecules must alter the conformation of the PC headgroup in such a way that the chains, following the change in headgroup orientation, are able to form the hexagonal undistorted lattice. This behavior is remarkable, because the fluid DOPtdIns monolayers possess no long-range lateral order. Furthermore, the headgroup of DOPtdIns is both large and negatively charged (phosphorester), neither of which is conducive to forming condensed and highly ordered films.

Effective phase separation in binary mixtures of DSPC and DOPtdIns(4,5)P_2

π/A isotherms of monolayers of DSPC, DOPtdIns(4,5)P_2, and their binary mixtures in different molar ratios (molar fraction of DOPtdIns(4,5)P_2 x_{PIP2} = 0.20, 0.25, and 0.35), measured at 20°C on 10 mM PB (pH 8), are shown in **Fig. 5** (left). DOPtdIns(4,5)P_2, with two unsaturated fatty acid chains, exhibits a fluid phase with low collapse pressure at approximately 30 mN·m^{-1}. The slope of the isotherm is extremely gentle, indicating either high compressibility of the film or a slow dissolution process. For the binary mixtures, the film becomes progressively more expanded with increasing molar percentage of DOPtdIns(4,5)P_2. If the compression reaches the collapse pressure of DOPtdIns(4,5)P_2, a slight break in the isotherm can be seen. Compared with the pure DOPtdIns(4,5)P_2 layer, the investigated mixtures exhibit much higher stability. Below 30 mN·m^{-1}, the slope decreases continuously with increasing amounts of DOPtdIns(4,5)P_2 because of the higher compressibility of the fluid part of the film. Above the collapse pressure of DOPtdIns(4,5)P_2, the slope of the mixed layers is similar to that of DSPC. The molecular areas determined in the binary mixtures of DSPC and DOPtdIns(4,5)P_2 as a function of the molar fraction of DOPtdIns(4,5)P_2 show a linear behavior until x_{PIP2} = 0.35 (Fig. 5, right). Based on the miscibility rule of liquid crystals, this can be taken as an indication of an almost complete demixing in the mixed monolayers. Comparing the measured and the linearly extrapolated molecular areas points at a noticeable solubility/instability of the DOPtdIns(4,5)P2 monolayer. In the mixtures with DSPC, the stability (decreased solubility into the subphase) of DOPtdIns(4,5)P_2 at the surface is enhanced.

The assumption of an almost completely phase-separated system is supported by the BAM images presented in **Fig. 6**. The mixed DSPC/DOPtdIns(4,5)P_2 monolayer (x_{PIP2} = 0.25) shows clearly the coexistence of two phases. At 40 mN·m^{-1}, a large amount of DOPtdIns(4,5)P_2 is assumed to be dissolved into the subphase (large deviations of the measured and extrapolated molecular areas), and therefore only small areas of a fluid phase are visible within the condensed DSPC phase.

The mixed DSPC/DOPtdIns(4,5)P_2 monolayers exhibit the same condensed phase structure as pure DSPC, with small changes in Bragg peak positions (Fig. 3, right column). If one compares the lattice parameters of the pure DSPC and the DSPC/DOPtdIns(4,5)P_2 mixture, slightly smaller tilt angles and unit cell dimensions can be found for the mixed monolayer. These changes indicate that a small amount of DOPtdIns(4,5)P_2 is incorporated into the condensed DSPC phase, leading to the observed structure changes. The slight tilt angle decrease can be observed up to high lateral pressures (Fig. 4). At low surface pressures, the effect of DOPtdIns(4,5)P_2 is only slightly less than or is similar to that of DOPtdIns at the same mole fraction. At pressures above 30 mN·m^{-1}, the difference between the two compounds is remarkable. Only DOPtdIns is able

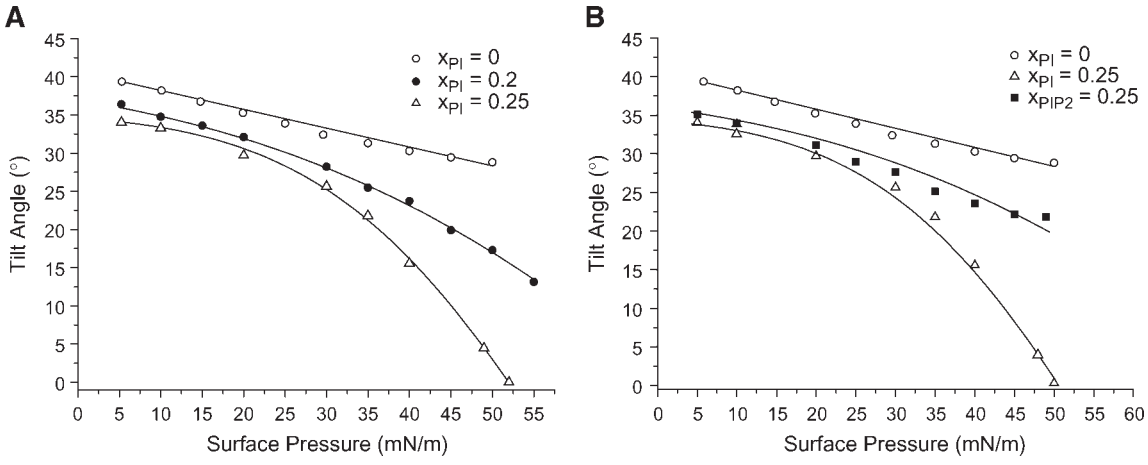

Fig. 4. Tilt angle versus surface pressure for DSPC and two different molar fractions of DOPtdIns in binary mixtures (A). Comparison of the tilt angle dependence of DSPC and two mixtures with DOPtdIns or DOPtdIns(4,5)P_2 with the same molar ratio (B).

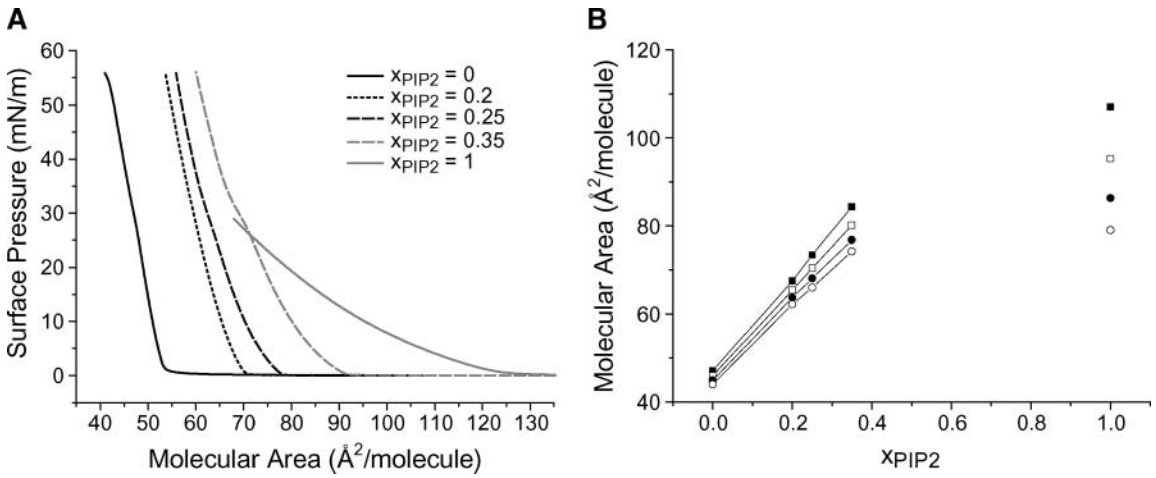

Fig. 5. A: π/A isotherms of DSPC (x_{PIP2} = 0) and selected DSPC/DOPtdIns(4,5)P_2 mixtures (mole fractions indicated) on 10 mM PB, pH 8, at 20°C. B: Molecular area in dependence on the molar ratio of DOPtdIns(4,5)P_2 in the mixtures at 5 mN·m^{-1} (closed cubes), 10 mN·m^{-1} (open cubes), 15 mN·m^{-1} (closed circles), and 20 mN·m^{-1} (open circles). The lines connecting the points represent the linear behavior for a completely demixed or ideally mixed binary system.

to induce a phase transition to the LS phase. Obviously, the miscibility behavior of the two compounds is completely different. Whereas DOPtdIns is, to a larger extent, miscible with DSPC, even at higher lateral pressures, DOPtdIns(4,5)P_2 is, at least above 30 mN·m^{-1}, mostly not miscible with DSPC.

DISCUSSION

Based on the presented results, DSPC/DOPtdIns(4,5)P_2 mixtures form condensed phase domains containing mainly DSPC and small amounts (not detectable by π/A isotherms) of DOPtdIns(4,5)P_2 surrounded by a fluid phase composed of predominantly DOPtdIns(4,5)P_2, perhaps containing a very small amount of DSPC. The small amount of DOPtdIns(4,5)P_2 that is mixed with DSPC decreases on compression because of the considerable solubility of DOPtdIns(4,5)P_2. DSPC containing a small amount of DOPtdIns(4,5)P_2 forms a slightly different monolayer structure (smaller tilt angle of the aliphatic chains) compared with pure DSPC. First of all, one must consider that the highly charged headgroup of DOPtdIns

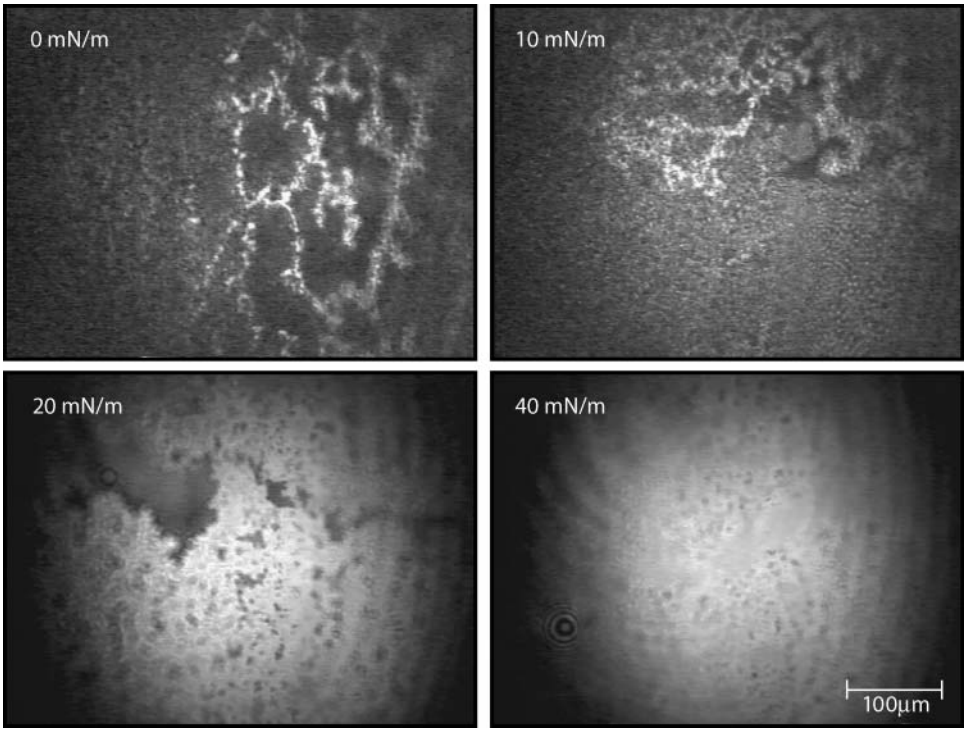

Fig. 6. BAM images of a 3:1 DSPC/DOPtdIns(4,5)P_2 (x_{PIP2} = 0.25) monolayer on 10 mM PB, pH 8, at 20°C. Surface pressures and scale bar are indicated. The corresponding isotherm can be found in Fig. 5.

$(4,5)P_2$ carries three negatively charged phosphate groups that lead, as long as they are fully ionized, to strong interactions with water molecules and ions in the subphase. Such interactions can stabilize the monolayer of highly charged compounds on the one hand, and induce demixing on the other hand. Such demixing has also been shown in experiments using mixed vesicles of phosphatidylinositol monophosphates and phosphatidylcholines. The mutual interaction of the phosphatidylinositol monophosphates is pH dependent and results in the formation of phosphoinositide-enriched microdomains (17). These microdomains are assumed to be stabilized by a hydrogen bond network, which utilizes the inositol ring hydroxyl groups as hydrogen donors, whereas the phosphomonoester, phosphodiester, and accessible hydroxyl groups in adjacent molecules function as acceptors.

Water can also play a crucial role in terms of shielding the strong repulsive forces within the headgroup region. In addition, the "water-shield" below the monolayer could be a buffering system that donates protons effectively (recruiting from the subphase below the ordered water layer) if the repulsion is getting too strong. It has been shown that densely packed molecules in highly ordered condensed monolayers are less protonated in comparison with fluid layers of molecules with the same headgroup (18). Therefore, it can be assumed that the ionization state of the phosphate residues of the lipid headgroup depends strongly on the molecular area of the phosphoinositide in the monolayer. The smaller the distance between like-charged phosphate groups is, the stronger is their electrostatic repulsion and the higher the pK. But this effect alone cannot explain the ability of the highly charged phospholipids to form DOPtdIns$(4,5)P_2$-enriched domains. Therefore, we assume that the environmental water plays an important role for the phosphorylated isoforms of the phosphoinositides and their distribution within the membrane. For the monolayer system, the water molecules are involved in the regulation of the complicated interplay of repulsive and attractive forces within phosphoinositide-containing lamellar structures. On the other hand, the buffer components are also able to shield the repulsion and can, because a divalent form is also available, act as a linker between the negative charges of the inositide headgroups. Both the water and the buffer components might be able to cause/enhance the demixing of DOPtdIns$(4,5)P_2$ in mixtures with DSPC.

According to the miscibility rules of liquid crystals (15), the two components forming different phases cannot be fully miscible. However, from a thermodynamic point of view, a small miscibility on both sides of the phase diagram can always be expected. Additionally, we have to discuss the monolayer preparation method. Both compounds are mixed in an organic solvent and co-spread at the buffer surface. The premixed compounds demix with certain kinetics. This demixing might be kinetically hindered, but should be completed in the time course of the experiments. However, a small amount of DOPtdIns$(4,5)P_2$ can obviously stay in the condensed DSPC phase, and vice versa, a few DSPC molecules can remain in the fluid DOPtdIns$(4,5)P_2$ phase. This could explain the increased stability of the mixed layers, compared with pure DOPtdIns$(4,5)P_2$.

The situation for the binary mixture of DSPC with the nonphosphorylated DOPtdIns is completely different. DOPtdIns is capable of mixing with the condensed DSPC to a much larger extent than DOPtdIns$(4,5)P_2$ is. The mixed condensed phase exhibits a structure that has never been observed in pure DSPC monolayers. The structural changes must be connected with the orientation of the phosphoinositol headgroups, leading to changes in the DSPC headgroups arrangement. Furthermore, it is known that the hydration layer around the already large PC head group increases the effective headgroup volume, further

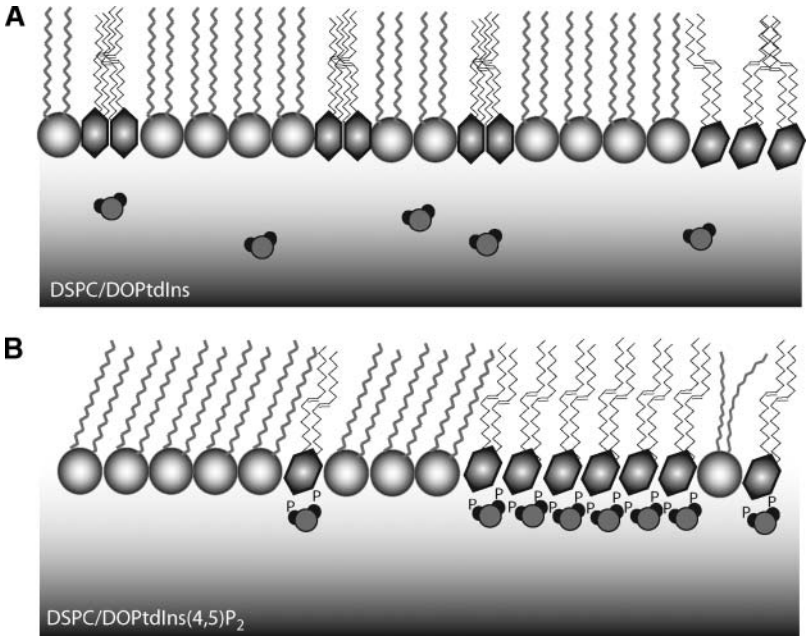

Fig. 7. Scheme of the molecular arrangement of the two phosphoinositides investigated in mixtures with DSPC. The nonphosphorylated inositides (DOPtdIns) are partly distributed within the condensed DSPC layer (**A**) and are able to change the orientation of the DSPC molecules. Oppositely, the highly charged DOPtdIns$(4,5)P_2$ is mostly phase separated (**B**). The water molecules interact with the DOPtdIns$(4,5)P_2$ and stabilize this phase.

leading to the strong mismatch in area requirement between head and tail. It has been shown that the tilt angle of DPPC (1,2-dipalmitoyl-phosphatidylcholine) is drastically reduced on ethanol/water subphases. This was explained by both a decrease of the hydration shell around the headgroup and a change of the headgroup conformation due to interactions with the alcohol/water mixture (19). For phosphatidylcholines, a headgroup orientation nearly parallel to the water surface is assumed (20). This orientation could change to a more vertical arrangement. Such a behavior was found for the binding of an enzyme (phospholipase A_2) to DPPC (D-enantiomer). The protein binding enforces a dehydration and reorientation of the PC headgroup (21). This process is highly cooperative, including at least 100 PC headgroups per protein molecule. Another possibility to reduce the tilt angle is the insertion of alkanes into the hydrophobic part of the monolayer (22). In this case, the effective headgroup area is not changed, but the alkanes are incorporated into the ordered lipid arrays, thus changing tail orientation and lattice structure. Another example of the upright orientation of condensed PC molecules induced by an additional compound is the mixing with phosphatidylglycerol or *n*-hexadecanol (23). In contrast to the mixture of DSPC-DOPtdIns presented here, the headgroups of the latter additives are quite small, much smaller than the PC headgroup. Therefore, it is likely that the polar headgroup of PtdIns could disrupt the dipole-dipole interactions between the PC headgroups, thus allowing the conformational changes required for the observed effect. **Fig. 7** proposes a molecular arrangement within the monolayers of the two binary systems.

The ability of the unphosphorylated inositide either to mix to a much larger extent than DOPtdIns(4,5)P_2 or to demix much less effectively is very interesting in terms of possible cellular function. If we assume a local accumulation by demixing of the phosphoinositide after phosphorylation, the lipid phosphorylating kinases become important in terms of triggering the distribution of phospholipids in a way that changes the ability of the lipids to interact with their ionic environment. Many PtdIns(4,5)P_2-interacting proteins require a locally accumulated lipid substrate. An enzymatic phosphorylation of phosphoinositides in mixed domains leads to a demixing process and cooperatively reorganizes the host lipid membrane. The observed effect suggests that the role played by phosphoinositides in signal transduction is related to their ability to induce or reduce long-range interactions in phospholipid mixtures.

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